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Effects of ^{60}Co gamma radiation on eggs of tasar silkworm, *Antheraea proylei* (Lepidoptera)

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Abstract

The radiosensitivity in eggs of tasar silkworm, *Antheraea proylei* (Lepidoptera) was studied by analyzing the frequency of embryonic deaths, larval survivability, moulting, larval durations, stage lethalities, growth of hatched larvae, green and dry cocoon weights. The embryonic lethalities expressed in LD_{50} value was found to be 13 Gy at 12 hours after oviposition. The larval lethality up to maturation of hatched larvae increase as the dose is increased in general. Whereas, survivality of hatched larvae from treated eggs with 10 Gy is even higher than control samples. The larval durations in treated samples also show excellent results in 10 Gy samples. Total larval durations are shorter in treated samples than normal samples. The larval growth generally retards when dose increases. The green and dry cocoon weights harvested from 11 Gy treated samples unusually show significant vigour than normal samples at 5% level of significance.

Key Words: Gamma radiation, eggs, silk worm

Introduction

According to latest scientific research, irradiation has immense potential and lots of applications. It can disinfect grain, inhibit sprouting in potatoes and onions, post pone ripening of fruits, wipe off pathogens from frozen seafood, prevent microbial contamination of species, and generally enhance the shelf life of most foods. Low dose of radiation can also help in the speedy growth of plants, early flowering and enhanced yields (source, In focus, JNU News, 1987). Gamma radiation is more homogeneous and of higher energy than an X-ray beam (Sparrow, 1961). Radiation studies in non mulberry silkworms are in juvenile stage. In the mulberry silkworm, *B. mori*, some works have been reported. Poor silk yielding oriental race of *B. mori* may be improved through induction of beneficial mutations (Tazima, 1968).

Gamma irradiation of the eggs of *Bombyx mori* with doses of 2.00-4.00 Gy increased hatchability by 2.4-7.2%, larval survival rate by 3.65-17.93%, mean cocoon weight by 0.42-6.98% and raw cocoon yield by 6.7-16.5% (Petkov *et al.* 1998). Application of five doses of gamma radiation ranging from 0.01 to 1 Gy at the stage of development of eggs in *Bombyx mori* achieved the greatest effect at a dose of 1 Gy which resulted in a significant increase in larval weight and silk glands by 21.96 and 30.14% resp., an increase in cocoon weight and shell by 11.11 and 9.76%, resp., and an increase in the length of silk filaments and weight by 22.96 and 22.53%, resp., over the control (Abdel-Salam *et al*. 1995). Irradiation of eggs favourably influenced the fecundity of emerged adults. Eggs (48- and 144-h-old) of *Bombyx mori* when exposed to gamma radiation, the cocoon weight of the 4th generation of insects reared from treated eggs was greater than that of insects from untreated eggs (Rao *et al.* 1994). Ravindra-Singh *et al*. (1990), when studying the effect of gamma radiation on the eggs of 3 polyvoltine strains (Hosa Mysore, C. Nichi and MY1 of *Bombyx mori* found that among the treated generation, Hosa Mysore was found to be the most sensitive, followed by C. Nichi and MY1 affecting the economic characters and suggested that irradiation may be a useful tool for exploring genetic variation in silkworms . With a view to improving the economic species of silkworm, *Antheraea proylei* (n=49), an attempt has been undertaken in the present study on the effects of ^{60}Co gamma radiation in eggs after 24 hours of laying.

Materials and Methods

Eggs of the tasar silkworm *Antheraea proylei*, were collected from local government farms. The eggs were disinfected by dipping in 3% formalin solution for 15 minutes and washed thoroughly with distilled water and then dried in the room. After 24 hours of laying, the eggs were irradiated with 0, 5, 7, 9, 10, 11, 13, 15, 17, 19, 20, 21 Gy from a ^{60}Co gamma irradiator in the Manipur University Campus. Three replicates of 50 eggs in each were irradiated with each dose of gamma ray. The irradiated eggs were then incubated at 25±1 degree Celcius, in a BOD incubator. Hatchabilities were recorded for each dose treatment. The larvae hatched out from treated eggs were transferred to *Quercus acutissima* bushes with new shoots and were covered with large nylon nets to protect them from enemies like flies, birds and rats. The number of dead larvae was recorded everyday and growth of the larvae was compared. Photograph was taken when the controlled larvae reached late larval stage.

Treated eggs were observed twice everyday at an interval of 12 hrs. Hatching started after 9 days in control and after 10 days of treatment in treated samples. Hatching continued for 2 to 3 days in both controlled and treated samples. Maximum number of hatching took place on the first day of hatching. Frequency of dead larvae per day was noted. Larval durations i.e. duration between two consecutive moultings and number of cocoons formed were also noted. Green and dry weights of cocoons formed were taken. Comparison of growth was carried out between the different treatments.

Evaluation of LD_{50} dose

The percent hatchability of the different samples of irradiated eggs of *A. proylei* was noted and plotted against the doses for calculation of LD_{50} dose for eggs.

The concept of the 50% lethal dose (LD_{50}) as an endpoint for scoring radiation death has been borrowed from the field of pharmacology. The 50% lethal dose, or LD_{50}, is defined as the dose of any agent of material that causes a mortality of 50% in the experimental group, within a specified period of time (Hall, 1978).

Silk percentage or silk ratio (SR)

This character is measured as the ratio of the shell weight to total cocoon weight expressed in percentage. The silk ratio was calculated by the following formula (Joshi and Misra, 1982).

$$\text{Silk Ratio (SR)} = \frac{\text{shell weight}}{\text{green cocoon weight}} \times 100$$

Effective rate of rearing (ERR)

This character measures the rearing performance of a line and is calculated on the basis of the proportion of cocoons harvested to total number of worms mounted expressed in percentage as follows-

$$\text{ERR} = \frac{\text{number of cocoons harvested}}{\text{number of worms reared}} \times 100$$

Results

LD_{50} for eggs

In controlled samples, 100% hatching was there, whereas in treated samples with 5, 7, 9, 11, 13, 15, 17, 19, 20 and 21 Gy, the treated eggs showed 89%, 83%, 78%, 67%, 56%, 33%, 17%, 6%, 0% and 0% hatching respectively. The mean values of embryonic lethality were plotted for the controlled and treated replicates (Fig. 1). From the curve it can be estimated that the LD_{50} for hatching lies around 13 Gy for 12 hrs. old eggs in *A. proylei*. Hatching was maximum on the first day of hatching both in controlled (9th-11th days) and treated (10th-11th days) samples.

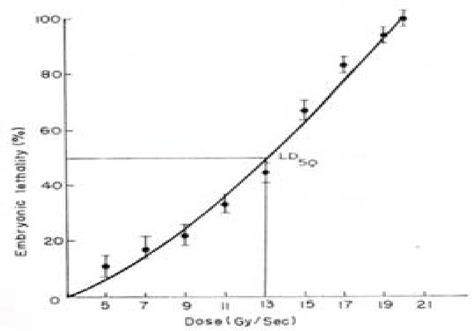

Figure 1: The embryonic lethality (%) in response to 0, 5, 9, 11, 13, 15, 17, 19 and 21 Gy on eggs with ^{60}Co gamma rays after 12 hr oviposition in the tasar silkworm, *A. proylei.*

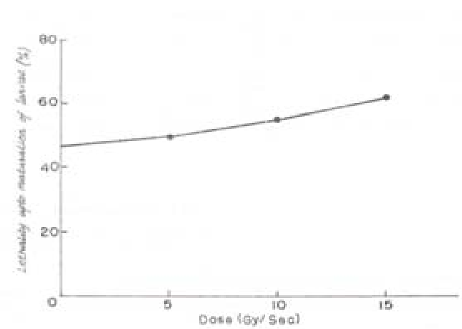

Figure 2:The lethality in percent upto maturation of larvae in treated eggs (12 hrs after hatching) of *A. proylei* with different doses of ^{60}Co gamma radiation.

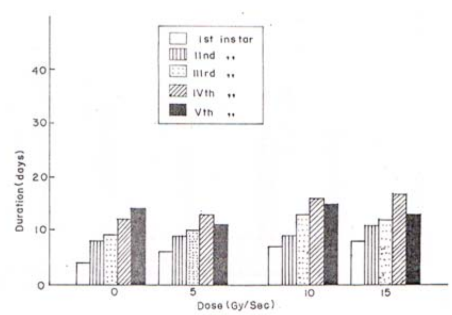

Figure 3:Effect of different doses of ^{60}Co gamma radiation treatment on larval duration of 12 hrs. old eggs after layiHHJJJgggng of the tasar silkworm, *A. proylei*

Larval survivality

The treated 12 hr old eggs gave rise to different percentages of hatchability. As the doses of treatment were very close to one another, only 0, 5, 10 and 15 Gy treated eggs were studied for larval survivality (Fig. 2). In 5 and 10 Gy treated samples, maximum number of larvae died in the IVth instar stadium and smaller frequency in Vth and IInd instar stadiums. In 15 Gy treated sample, maximum frequency of larvae died in the IInd instar stadium, while smaller frequencies died in the Vth, the IVth and the Ist instar stadiums and no mortality in the IIIrd instar stadium. None of them died in the Ist instar stadium in 0, 5 and 10 Gy. In the controlled sample, 20% of larvae died in the Vth instar stadium and 13% and 14% each in IInd and IVth instar stadium and none in Ist and IIIrd instar stadiums. The mortality in controlled samples was also reported from several tasar farms in Manipur. The larval lethalities reflect the lethality up to maturation of larvae (Fig. 3).

As we observe from figure 3, lethality up to maturation of larvae i.e. total larval lethality gradually increases parallel with dose treatments. Ultimately survivality up to maturation of larvae are 53%, 50%, 45% and 3% in 0, 5, 10 and 15 Gy treated eggs (Table I). Here the percentage is taken out of the total number of eggs treated. On the other hand, the survivality up to maturation of larvae were 63%, 56%, 70% and 40% in 0, 5, 10 and 15 Gy treated larvae respectively (Table II). Here percentage is taken out of hatched larvae. Here the survival frequency of hatched larvae is larger in 10 Gy treated samples than in controlled samples.

Table I: Survivality of treated eggs (12 hr old) upto maturation of larvae in *A. proylei.*

Dose (Gy/sec)	Survivality upto maturation of larvae (%)
0	53
5	50
10	45
15	38

Table II: Survivality of hatched larvae from treated eggs (12 hrs old) upto maturation of larvae in *A. proylei.*

Dose (Gy/sec)	Survivality upto maturation of larvae (%)
0	63
5	56
10	70
15	40

Table III: Weight (in gm) of sex specific green cocoon and cocoon shell of 11 Gy treated 12 hr old eggs of *A. proylei.*

	Male green cocoon (gm)	Female green cocoon (gm)	Male cocoon shell (gm)	Female cocoon shell (gm)
Controlled	3.131 ± 0.51	4.10 ± 0.86	0.28 ± 0.06	0.27 ± 0.05
Treated	4.75 ± 0.14	5.87 ± 0.56	0.51 ± 0.06	0.48 ± 0.09
Level of significance between controlled and treated at 5% level of confidence	S	S	NS	NS

Table IV: Silk ratio (SR) of sex specific cocoon of 11 Gy treated 12 hr. old eggs *of A. proylei.*

	Male	Female	Mean
Controlled	9.01 ± 2.34	6.97 ± 1.40	7.99
Treated	10.64 ± 0.99	8.15 ± 0.73	9.40
Level of significance between controlled and treated at 5% level of confidence	NS	NS	

Moulting and larval duration

Observations of successive moultings in controlled and treated egg samples reflected that radiation effect persisted on different instarwise larval stages (Fig. 4). Instarwise larval durations are the time taken in each instar stadium. Most of the larvae died in the moulting process. The process itself took more time to complete in treated samples. As a result the larval durations were elongated in treated samples.

Silk output

Gamma radiation also produces its effect on the silk output of male and female individuals. Among the doses given, vigorous crops were harvested from 11 Gy treated samples. Green cocoon weights were taken just after the harvest and silk shell weight were taken after emergence of moth and dissection of pupae. Green cocoon weight of controlled male was found to be between 2.51 gm. to 3.91 gm. And their mean value and standard error were calculated from every individual (Table-III). Female green cocoon weights of controlled sample was found to be between 2.69 gm to 5.84 gm and their mean value and standard deviation were calculated as 4.10 ± 0.86. The silk shell weights of controlled male and female were 0.28 ± 0.06 and 0.27 ± 0.05 respectively. Where the male and the female green cocoon weights of 11 Gy treated eggs were 4.75 ± 0.14 and 5.87 ± 0.56 respectively in terms of gm. And the male and female cocoon shell weights of 11 Gy treated eggs were found to be 0.52 ± 0.06 and 0.48 ± 0.09 respectively in gm. Using student 't' test at 0.05 level of confidence, the significance tests were evaluated. The green cocoon weights of both male and female treated samples were found to be significant, but the cocoon shell weights were observed to be not significant. The silk ratios (SR) of sex specific cocoons of 11 Gy treated eggs were also calculated (Table IV). The mean SR values were tested for significant difference using 't' test. The values were not significantly different at 5% level of confidence between the controlled and treated samples.

Discussion

The present study aims at evaluating the effects of gamma radiation on the developmental and commercial characters of the economic hybrid species of tasar silkworm, *A. proylei* to draw the degree of radioresistance for commercial utilization. Radiosensitivity of an organism is usually the range of doses causing the animal to die with haemopoietic syndrome phenomena (Yarmonenko, 1988). The radioresistance of various populations in a community can be predicted on the basis of laboratory studies. For example, the lethal exposure for most mammals lies between 200 R and 1000 R (2-10 Gy), and for insects it lies between 1000 R and 100,000 R (10-1000 Gy) (Casarett, 1986). Among vertebrates, mammals are generally more radiosensitive than birds, amphibians and reptiles. Most invertebrates have been shown to be more resistant than vertebrates (Bacq and Alexander, 1961). The degree of radiosensitivity varies greatly within a single species (individual radiosensitivity), while for a definite individual, it depends on the age and sex. Moreover, wide variation in radiosensitivity in different cells and tissues is observed in the same organism in which sensitive tissues coexist with radioresistant ones (Yarmonenko, 1988). Mortality is enhanced with higher doses of radiation in cockroach, *Periplaneta americana* (Wharton and Wharton, 1959) and in housefly, *Musca domestica* (Nelson et al., 1970). A dose of 2 kilorad (20 Gy) beta radiation inhibits the oothecal formation (Wharton and Wharton, 1957) and stunted ovaries development (Nelson et al., 1970). Irradiation of insects larvae leads to lethality, delay in pupation, developmental abnormalities, failure of emergence and imaginal death soon after eclosion (Ducoff, 1972). Nymphal development, reproductive capacity and mating competitiveness of *Dysdercus koenigii* (F), a pest of malvaceae plants also showed declining radiosensitivity with age when exposed to ^{60}Co Gamma radiation (Jayaraman and Mukherjee, 1980). Dose dependant pupariation delay and abnormal shape of puparia with higher doses of gamma radiation were reported in housefly, *Musca domestica* (Srinivasan and Kesavan, 1979). Survival or mortality curves are used for the quantitative studying of the radiosensitivity of an organism. The high frequency death of larvae in the moulting state might be due to the loss of mitotic index which is an inescapable phenomenon in moulting. Mitotic index is the P.C. of the number of dividing cells divided by the total number of cells. The increased survivality of hatched larvae in 10 Gy treated samples might be due to vigour obtained from radiation as it has even higher survival frequency than control.

LD_{50} value and resistance

The LD_{50} values are important data for the assessment of radioresistance of the animals. The LD_{50} value of 12 hrs. old eggs of *A. proylei* found to be around 13 Gy indicates that the radioresistance is very high in this organism. The $LD_{50/30}$ values for sheep, rabbit, rat and desert mouse were found to be 1.55 Gy, 8.4 Gy, 9 Gy and 15.20 Gy respectively (Hall, 1978). The molecular rationale for this radioresistance in insects is believed to involve very efficient DNA repair processes (Koval, 1980) which allow them to maintain their genetic integrity. It is generally acknowledged that dose rate has a significant effect on the LD_{50} in mammals (Chee et al., 1979). *A. proylei* is more radioresistant than *B. mori*. The LD_{50} for *B. mori* eggs, a few hr after oviposition, was 800R (=8 Gy); the eggs became more resistant with the progress of embryonic development reaching a plateau at day 7 when LD_{50} was as high as 6500R, both in non-dormant and dormant eggs (Akita et al., 1965). Lepidoptera are more radioresistant than other insect groups like Hemiptera, Diptera and Homoptera (Koval, 1983). Recent studies on the TN-368 lepidopteran insect cell line have suggested that the cells of insects have an intrinsic radioresistance. The mitotically active cells of this line are approximately 50-100 times more radioresistant than mammalian cells (Koval, 1983). In another view, the radioresistance of lepidopteran cells has been attributed to be a consequence of their possession of holokinetic chromosome; Murakami and Imai, (1974). Holokinetic implies that kinetochores or centromeres are spread out or diffused along the chromosome (White, 1973). The higher silk output in 11 Gy treated samples indicate that gamma radiation has useful effects i.e. vigour on low doses which may be useful in large scale productions in sericulture farms in our country.

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